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ATPase assay of purified, reconstituted CFTR protein[1]

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Abstract

The Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) is a phosphorylation and nucleotide regulated chloride channel. CFTR also directly mediates the hydrolysis of ATP and this catalytic activity is loosely coupled to CFTR channel gating. However, mechanistic detail regarding the role of ATP hydrolysis in channel function is lacking. Our further understanding of the molecular basis for normal channel activity requires kinetic analysis of the ATPase activity by the full-length protein. This article describes an effective assay of ATPase activity by purified, reconstituted CFTR protein.

Keywords: Catalysis; Phosphorylation; Turnover number

1. Introduction

The chloride (Cl^-) channel activity of phosphorylated CFTR is regulated by nucleotide interaction with its two nucleotide binding domains (NBDs); NBD1 and NBD2 and ATP hydrolysis [1–9]. To date, CFTR is the only ion channel to possess measurable ATPase activity and this property has galvanized ion channel physiologists to study the potential link between this enzymatic activity and CFTR channel gating.

In-depth studies of the coupling mechanism between CFTR ATPase activity and channel gating require direct assessment of the kinetics of both functions. However, such experiments are challenging as CFTR exhibits a relatively low rate of ATPase activity. Therefore, to measure the ATPase activity of CFTR accurately, it is essential to eliminate the ATPase activity contributed by other proteins. Thus, ideally, CFTR ATPase activity should be measured using purified protein. Channel gating by purified, reconstituted CFTR can be assessed simultaneously in planar lipid bilayer studies.

Abbreviations: NBD, nucleotide binding domain; PFO, pentadecafluorooctanoic acid.

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[1] Adapted from Kogan et al. Methods in Molecular Medicine 2002;70:143–57.

2. ATPase measurements

In our studies, we found that CFTR is a slow ATPase, exhibiting a low catalytic rate of 0.2 ATP molecules hydrolyzed per second [10]. This finding was recently reproduced by Aleksandrov et al. [11]. However, calculation of the actual turnover number requires an accurate assessment of the quantity of functionally reconstituted protein. We estimated on the basis of planar lipid bilayers that 10–20% of CFTR molecules were functionally reconstituted as a Cl^- channel [12]. Therefore, assuming that only 10% of the CFTR protein is functionally reconstituted, the turnover rate of the hydrolytic activity of CFTR may be as high as 1–2 ATP molecules per second, a rate still considerably slower that other ATPases including the related ABC protein, *P*-glycoprotein [13].

Detailed procedures describing purification of CFTR-His proteins in the presence of the fluorinated surfactant pentadecafluorooctanoic acid (PFO) and reconstitution into phospholipid liposomes are published elsewhere [14]. Similarly, detailed methods for the measurement of CFTR ATPase activity have been published previously and appear in the online Virtual Repository [15]. Briefly, radioactive, [α^{32}-P]-labelled ATP is allowed to react with freshly purified CFTR protein, reconstituted into proteoliposomes in the presence of 20 mM $MgCl_2$, buffered saline and varying concentrations of cold ATP. Empty proteoliposomes (with no CFTR) are used as the control. After the

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2-h incubation period and the subsequent termination of the reaction, the ratio of "hot" ADP to "hot" ATP is used to assess intrinsic ATP hydrolysis by CFTR. ADP is separated from ATP by thin-layer chromatography and visualized by phosphoimager.

In order to measure ATPase activity, it is essential that freshly purified and reconstituted CFTR be used in the assay. The conditions for optimizing the stability of purified CFTR have not yet been determined. In our experience, it is preferable to maintain the reconstituted protein at 4 °C as freezing completely abrogates function. However, even at 4 °C, ATPase activity is reduced dramatically by 1-week post-reconstitution relative to that activity measured immediately after reconstitution. Furthermore, the activation of CFTR ATPase activity by PKA phosphorylation, a modification which usually increases activity by twofold to threefold in freshly reconstituted protein, diminishes with time after reconstitution. As yet, the physical basis for this loss in activity remains unclear and remains a major impediment to structural studies on the protein. Importantly, within 2–3 days after purification and reconstitution, the magnitude and regulation of CFTR ATPase activity shows consistency.

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